



# PREPARATION AND EVALUATION OF DARK CHOCOLATE WITH PALM JAGGERY

Pandge Vaishnavi Rajkumar

Student

Aditya pharmacy college beed

## I. ABSTRACT

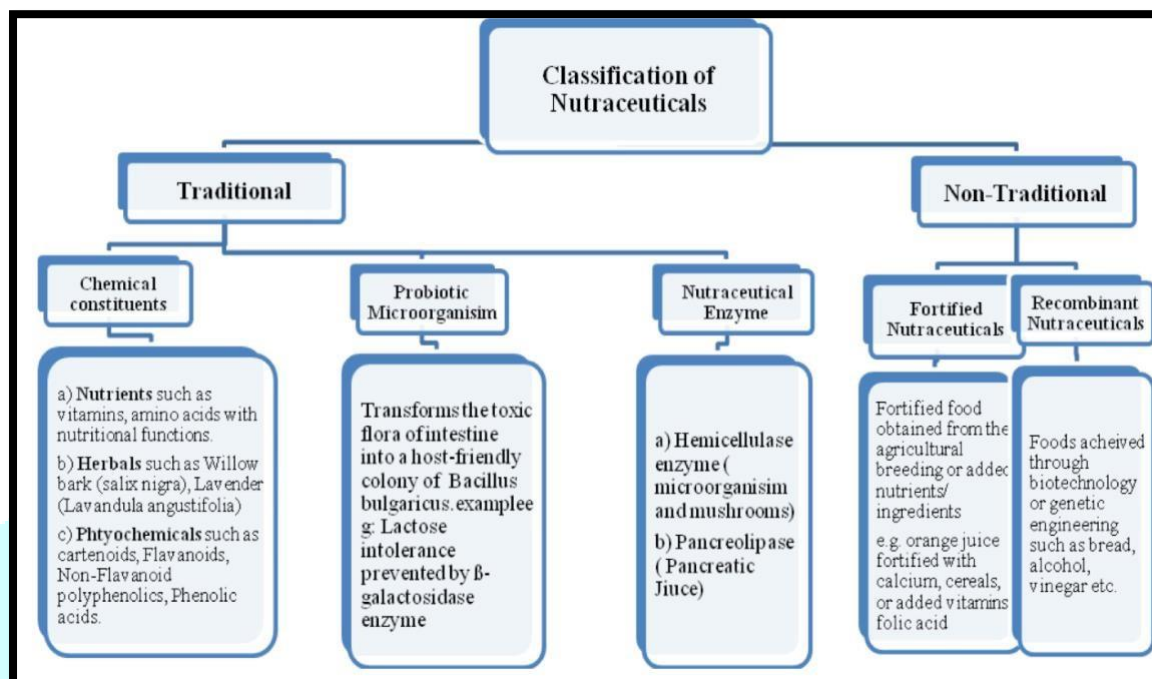
Borassus flabellifer is Greek word commonly known as Palmyra palm. Neera is unfermented toddy obtained from palmyra trees. Many of the people are consuming the sap in the form of neera or toddy. Palm jaggery is a sugar rich and high medicinal properties product. There is a huge demand in the market for high nutritive products made with jaggery in place of refined sugar. This study was conducted to develop palm jaggery based chocolates. The palm jaggery chocolates-based chocolates was prepared with different proportions of palm jaggery, i.e., 50% respectively. These developed palm jaggery chocolates were acceptable with good flavour and desirable textural properties.

## II. INTRODUCTION

### 2.1 NUTRACEUTICALS

#### 2.1.1 CATEGORIES OF NUTRACEUTICALS

1. **Nutrient**: A feed constituent should be available in the form and given at a level that will help support the life of an animal. Some of the feed nutrients are proteins, fats, carbohydrates, minerals and vitamins.
2. **Dietary Supplement**: A product that contains one or more of the following dietary ingredients: vitamin, mineral, herb or other botanical, amino acid (protein) and also includes concentrates, constituents, extracts or metabolites of these compounds.
3. **Nutraceutical**: Any non-toxic food component that has scientifically proven health benefits, including prevention and treatment of disease.
4. **Herbals**: Herbs or botanical products are used as concentrates and extracts that provide various remedies to treat acute and chronic diseases.



**Figure 1:- FLOW CHART OF CLASSIFICATION OF NUTRACEUTICALS**

### 2.1.3 BENEFITS OF NUTRACEUTICALS

From the consumers' point of view, functional foods and nutraceuticals may offer many benefits:

- May increase the health value of our diet.
- May help us live longer.
- May help us to avoid particular medical conditions.
- May have a psychological benefit from doing something for oneself.
- May be perceived to be more "natural" than traditional medicine and less likely to produce unpleasant side-effects
- May present food for populations with special needs (e.g., nutrient-dense foods for the elderly).

## 2.3 COMPOSITION OF RAW PALM JAGGERY/ NEERA

**Table 1: The chemical percentage composition of Neera varies, depending on various factors. Substance And Concentration (g/100 mL)**

Total solids(g/100ml)	15.2-19.7
Specific gravity	1.058-1.077
Original reducing sugar(g/100ml)	5.58
Total ash(g/100ml)	0.11-0.41
Alcohol in %	Nil
Phosphorus (g/100ml)	7.59
Total Protein(g/100ml)	0.23-0.32
Ascorbic acid(mg/100ml)	16-30
Total reducing sugar (g/100ml)	9.85
Citric acid(g/100ml)	0.50
Ph	3.9-4.7
Iron (g/100ml)	0.15
Total sugar(g/100ml)	14.40
Sucrose	12.3 - 17.4
Protein	0.23 -0.32

**Table 2: Biochemical and mineral composition of freshly collected Neera .**

### Biochemical parameters Range

Ph	6.57-7.50
Total sugar (g)	10.08-14.50
Reducing sugar (g)	0.439-0.647
Amino acids	0.123-0.338
Sodium (mg)	69.4-117.5
Potassium (mg)	146.1-182.4
Phosphorous (mg)	2.0-6.4
Manganese (mg)	0.009-0.014
Copper (mg)	0.028-0.035
Zinc (mg)	0.018-0.026

## 2.4 NUTRITIONAL BENEFITS OF PALM JAGGERY

- It is translucent, high in nutritional value and susceptible to natural fermentation at ambient temperature within a few hours of extraction. On fermentation, neera becomes toddy.

- It is widely consumed in India, Sri Lanka, Africa, Malaysia, Indonesia, Thailand, and Myanmar. It is a delicious and nutritious drink rich in carbohydrates with sources of minerals, vitamins proteins etc and nearly neutral pH.
- It contains ascorbic acid, nicotinic acid and riboflavin.
- Neera is popular as a health drink on account of its high nutritive value, delicious taste and agreeable flavour.
- Neera is otherwise called sweet toddy, a sap extracted from the inflorescence of various species of toddy palms.
- It is a natural and nonalcoholic beverage, high in nutritional value and an instant thirst quencher.
- It is sweet, oyster white, and translucent.
- This sweet sap of the palm is fast becoming a popular drink on account of its highly nutritive value, delicious taste and agreeable flavour.

## **2.5 USES OF PALM JAGGERY**

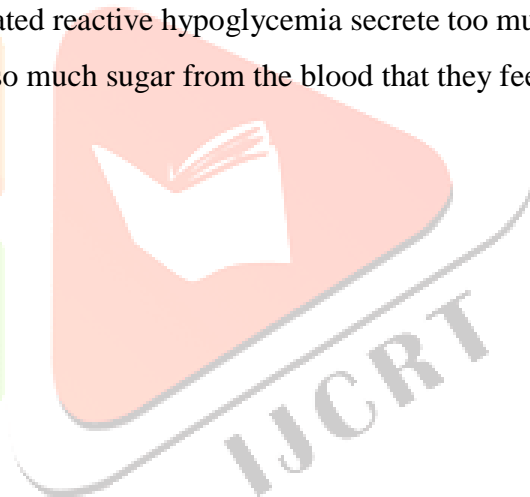
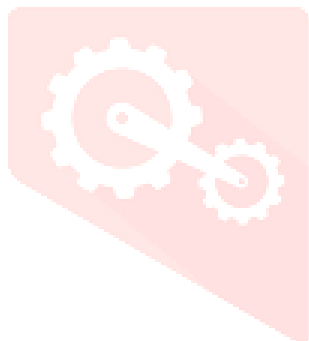
- Uses of neera Neera is popular as a delicious healthy drink.
- It can be promoted as an instant energy provider, as a functional food or nutraceutical drink
- The nutrient-rich "sap" has low Glycemic Index (GI of only 35) and hence diabeticfriendly since very low amounts of the sugar is absorbed into the blood.
- It is an abundant source of minerals, 17 amino acids, vitamin C, broad-spectrum B vitamins, and has a nearly neutral pH.
- It is good for persons in post-operative care due to a high content of electrolytes
- It is a body cooler and is good for digestion and with no side effects

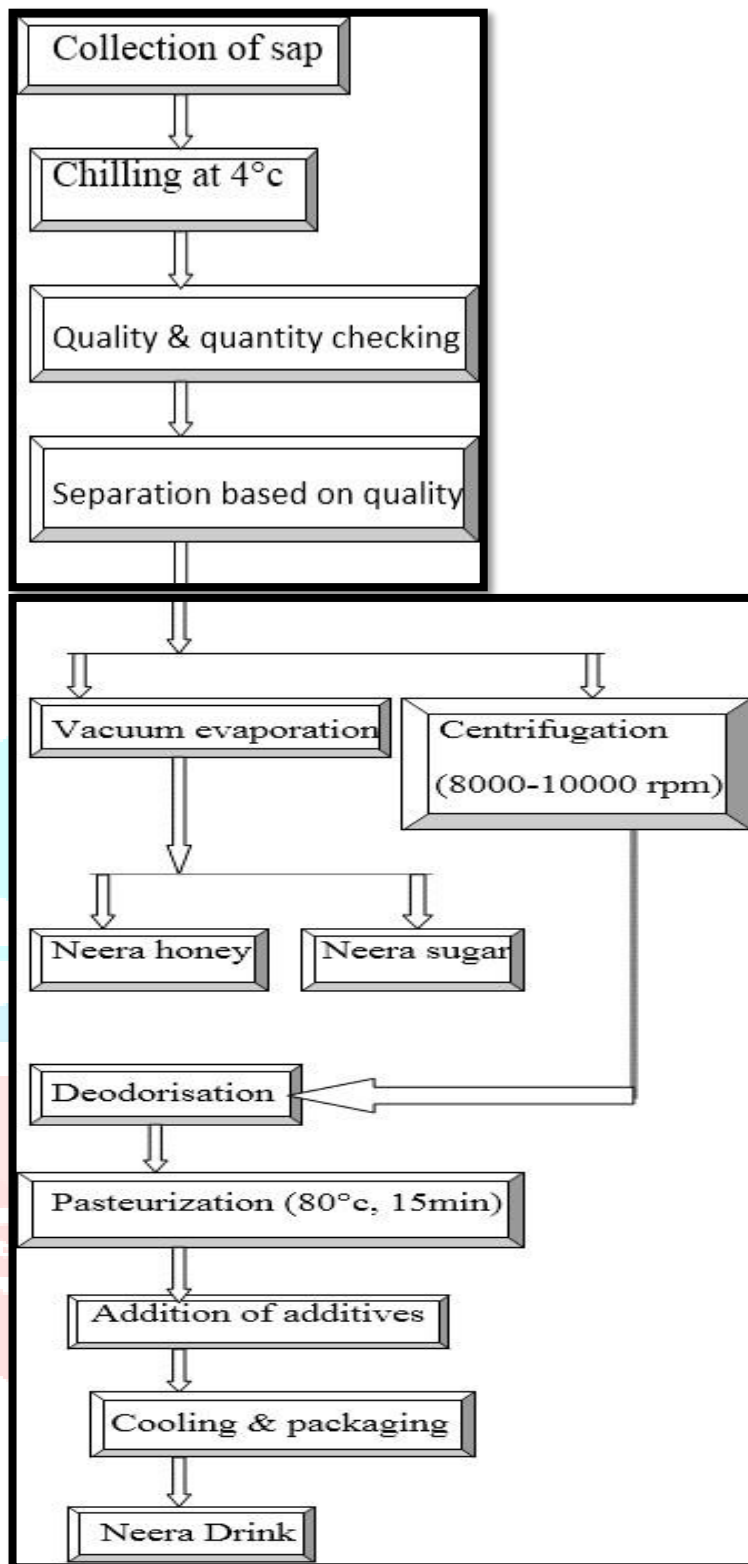
### **2.5.1 Some Good Effects of Neera**

- It is wholesome, cool & good for improving the health.
- Supplement for iron & vitamin deficiency.
- Clinical studies indicate medical applications for-asthma, tuberculosis, bronchial suffocation & piles.
- Believed to facilitate clear urination and prevent jaundice.
- High amount of glutamic acid which is the amino acid used by the body to build proteins.
- High in inositol which beneficial for the treatment of eye abnormalities, eczema etc.
- **Benefit of taking low GI food:** -By helping to maintain lower blood sugar insulin level, a low-GI diet may be useful in preventing and treating a variety of health problems. Here are some examples of how eating low on the Glycemic Index can help promote excellent health:

- **Diabetes** – Substituting low - GI carbohydrates (like thick – cut oats, pasta and legumes) for high-GI carbohydrates (like processed cereals, white bread, and potatoes) can help lower blood glucose levels in people with diabetes. This is why the GI has been an integral part of medical nutrition therapy for diabetes in Australia, New Zealand, Canada and Europe for many years.
- **Cancer** - Insulin is a cellular growth factor; many studies have shown an association between high insulin levels and a variety of cancers including breast, colorectal, prostate, and pancreas. Other studies have shown links between diets high in sugar, refined carbohydrates, Glycemic Load and cancer. This suggests that lifestyle changes like maintaining a healthy body weight, exercising and eating a healthy low – GI diet may help protect against cancer at least partly by lowering insulin levels.
- **Cardiovascular Disease** - As with type 2 diabetes, researchers have found that a diet rich in refined and high GI carbohydrates may substantially raise the risk for heart disease. These foods increase blood insulin levels which in turn contribute to high blood pressure, higher levels of blood fats (triglycerides), lower levels of HDL (good) cholesterol and an increased tendency for dangerous clots to form and linger in the blood.
- **Hypoglycaemia** - People who have meal related reactive hypoglycemia secrete too much insulin after eating. This causes the cells to remove so much sugar from the blood that they feel weak, irritable, giddy and hungry.

## **2.6 NEERA PRODUCTION**





**Figure 2 :- MANUFACTURING PROCESS OF NEER**

### 2.6.1 COLLECTION OF NEERA

- Neera is collected every morning just at sunrise because as soon as the sun light hits the surface the very process of fermentation starts.
- The sap is extracted and collected by a tapper.
- The sap is collected from the cut flower of the Coconut.
- A container is fastened to the flower stump of collect the sap.
- The white liquid that initially collects tends to be very sweet and non- alcoholic.

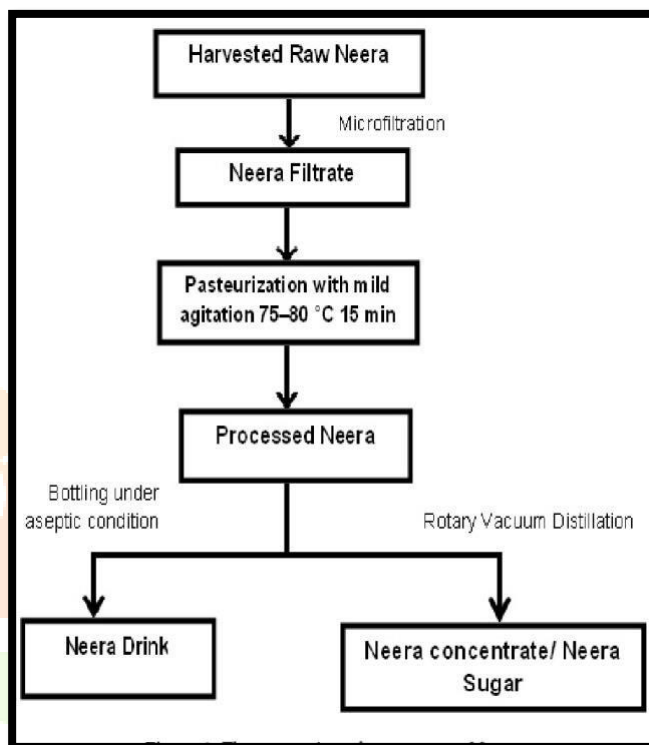
- Neera is brought down from the top of the tree in either earthen pots or vessels, and then poured into stainless steel containers and bigger vessels, after being filtered through a fine mesh cloth or wire-mesh.
- Neera is very much susceptible for fermentation at room temperature so after collection it will kept at low temperature & treated with some preservatives.

## 2.6.2 TAPPING AND HARVESTING

The harvesting by the traditional tapping practiced by the toddy tappers

1. The selection of healthy palms: As the step of tapping, 250 healthy coconut palms (*Cocos nucifera*) of approximate 30 m with mature unblossomed spadix. height of the tree, number of mature spadix and general health of the palms taken as the criteria for selection.
2. Surface sterilization of the palm crown: the tapping process, traditional toddy tappers were identified and trained.

Unlike the traditional tapping **Figure PREPARATION OF NEERA SUGAR**



expose the spadix for the smooth tapping. As the first step of Neera harvest, a mild disinfectant—0.05% sodium hypochlorite—was sprayed in the crown to create an aseptic environment.

3. Cleaning of the spadix: The identified spadix for tapping was sprayed with distilled water from the base to the top for cleaning. After washing, the surface of spadix was cleaned by tissue paper to make it dry.
4. Beating of the spadix: The cleaned spadix was subjected to an initial beating process from the base to the top with a professional skill for 3–4 days. After the initial beating, the tip of the spadix was chopped with a sharp sterile knife—the indigenous tool of the traditional tapper. The beating process was continued for 10–15 days. Every day, the chopped end of the spadix was covered by a sterilized plastic mesh carefully after the beating. This was for preventing entry of insects and other small organisms. The beating was done twice—in morning and evening.
5. Application of the sterilized clay on the spadix: The excised end of the spadix always appeared as wet due to the exudation of Neera from the spadix during the beating process. There is every possibility of leaching of this sugary exudate to the basal part of the spadix which will damage the



entire system. This can be prevented by spreading a sticky matrix on the surface of the cut end. Traditionally toddy tappers were using sticky natural clay on the surface. In the case of Neera harvesting, slight modification was made by sterilizing the clay, i.e., sterilized clay was applied on the cut end of the spadix by the tapper. The tapper should wear gloves while applying the clay so that further contamination can be avoided. The clay was sterilized by autoclaving.

6. The collection vessel: Traditionally a clay pot was used for the collection by toddy tappers. Due to the repeated use of the pot by the tappers, for the collection, it was not properly maintained in a hygienic way. In the case of Neera harvesting, an aseptic mode was created by using a sterilized plastic vessel of 5 L capacity. The vessel was cleaned and sterilized prior to the insertion to the processed spadix for collecting Neera.
7. Insertion of the sterile vessel: The vessel was inserted to the spadix after 15–20 days of beating based on the flow of Neera from the spadix. After 15–20 days, Neera was exudated from the processed spadix at a rate of 100–200 mL initially and the volume was increased to 1 L after one month and subsequently to 2 L after two months. The volume of the Neera exudate varied from palm to palm based on several factors—age, height, health of the palm and time of collection.
8. Collection of neera

### **2.6.3 PREPARATION AND USAGE OF THE ANTI-FERMENTATION**

#### **SOLUTION (AFS)**

- A new combination was prepared for suppressing the tendency of fermentation of raw Neera during new combination was prepared for suppressing the tendency of fermentation of raw Neera repeated field trials for optimizing the anti-fermentation formula.
- Raw Neera exudated from the coconut spadix during harvesting has got the innate tendency of getting fermented.
- Therefore, it is essential to prevent the auto-fermentation by suppressing the growth of microbes prior to processing.
- An anti-fermentation solution (AFS) was prepared by the combination of two chemicals—citric acid (CA) and potassium metabisulphite (KMS)—that have been accepted as preservatives in food and natural drinks.
- The combination of the two salts was optimized to the level of 4–5 mM for citric acid with a concentration of KMS 1.5–2.5 mM in raw Neera/L. Using the treatment of the anti-fermentation solution in the harvested Neera, yet another problem appeared that was the fermentation of raw Neera at the crown within the collection vessel during harvesting.
- Though traditionally the toddy tappers have been practicing the usage of calcium hydroxide (lime) in the vessel for collecting sweet toddy, the presence of lime remains as a health hazard.
- Moreover, the processing steps of removal of the lime look more tedious. Therefore, the fermentation of Neera within the collection vessel was checked by adding a concentrated volume of 10 mL AFS/L Neera with the same molarity (5 mM CA and 2 mM KMS) to the collection



vessel at the time of insertion by the tapper. Based on the volume of Neera exudate from each palm, the volume of AFS can be adjusted by the tapper.

### ○ Processing and Storage of Harvested Neera

The harvested Neera was filtered at the plantation itself through a cheese cloth for removing the solid debris. The filtered Neera was stored in a can and transported to the laboratory for further processing. Prior to the processing of the raw Neera, the brix value and the pH of the harvested Neera were checked. The processing steps include

- Microfiltration,
- Pasteurization,
- Bottling and
- Storage.

- (i) **Microfiltration:** The raw Neera was filtered through micro filters with a pore size of 100 microns under aseptic conditions.
- (ii) **Pasteurization:** The filtered raw Neera was stored in a sterilized vessel for pasteurization for 10–15 min at a temperature range of 75–85 °C. A mild agitation was inevitable during pasteurization. The temperature was kept constant at 80 °C during pasteurization by remote control.
- (iii) **Bottling:** The pasteurized Neera was bottled by an automatic bottling machine with a volume (200 mL) in polypropylene (PP) bottles and 300 mL in glass bottles and capped and sealed automatically. The entire steps were done in a closed system for avoiding further contamination during processing.
- (iv) **Storage:** Shelf life was checked periodically by analyzing the pH, brix value and nutritional components

### 2.6.4 QUALITY ANALYSIS OF NEERA

As the first part of the analysis, the Neera was subjected to the quantification of the microbial load at three levels:

- a) Raw Neera at the time of harvest,
  - b) processed Neera and
  - c) processed Neera after 3 months of storage.
- The total fungal count was estimated by following the protocol of the Indian Standard: 5403 test method.
  - A yeast extract-dextrose-chloramphenicol-agar medium was prepared, and pour plates were made using the raw, processed and stored Neera samples at different dilutions.
  - The plates were then incubated at  $25 \pm 1$  °C for 3 days.
  - A control plate was also maintained. The total bacterial count was detected following the IS: 5402 test method.
  - The yeast extract-glucose-casein agar medium pour plates were prepared using the raw, processed and stored Neera samples at different dilutions.

- They were incubated at  $30 \pm 1$  °C for 72 h. A control plate was also maintained.



## **IV.SCOPE AND OBJECTIVES**

### **4.1 SCOPE: -**

Normal sugar is a major contributing factor in the aggravation of diabetes, heart disease, gum disease, obesity, and other health problems. As a result, many people have been turning to "natural" sweeteners as a healthier choice. Globally, neera and its value-added products are being manufactured by all the major coconut producing countries except India.

Neera is diabetic-friendly due to low Glycemic Load / Glycemic Index. Neera is rich in minerals and vitamins and it contains glucamic acid necessary for proteins synthesis. It aids in digestive health. Neera contains vitamins (Vit. A and Vit. C), which have antioxidant properties thereby preventing damage or death of cells. Neera can be promoted as a health drink, instant energy provider, functional food and nutraceuticals. It is good for post-operative care due to the high content of electrolytes. [8]

### **4.2 OBJECTIVES: -**

1. To prepare dark chocolate with palm jaggery
2. To discuss the composition of palm jaggery.
3. To study the uses and benefits of palm sugar.
4. To study nutritive properties of neera.
5. To examine the polarimetric test and uv spectroscopy of palm jaggery.
6. To analyse the Rheological basic tests of neera jaggery.



**Figure 4 DARK CHOCOLATE PREPARED IN LABORATORY**

## V. MATERIALS AND METHODS

### 5.1 Raw materials

Cocoa Dark Chocolate were purchased and dates powder was obtained from local shop from Market. (Lonavala). while different kinds of palm sap-based sugars (coconut sugar and palm sugar) were purchased from amazon online shopping

### 5.2 Sample preparation

Dark chocolates with a total fat content of 36% were prepared according to the following formulation: 49.0% palm sugar, 21% cocoa mass, 17% cocoa butter and 0.4% xanthan gum. Specification of sugar tested are sugar composition, glucose and sucrose were determined by refractometer and polarimeter

### 5.3 Chocolate processing

Chocolates were produced using a combination of mixing and ball milling. The production consisted of three steps, namely mixing refining and liquefaction mixer.

### 5.4 Tempering and Moulding

Tempering was performed manually followed by measuring the temper index (TI) through a temper meter to verify that the chocolates were tempered correctly ensuring contraction, desired gloss and snap. All the chocolates had TI in the range of 4 to 6 and a chocolate temper unit (CTU) of 22.5-24.60 C, both indicative for well-tempered products. Afterwards, tempered chocolates were poured into moulds of 100 mm x 24 mm x 10 mm and subsequently cooled at 12°C for 1 hour. Afterwards, the chocolates were de-moulded and transferred to a thermal cabinet at 20°C for 24 hours.



**Figure 5:- PALM JAGGERY CHOCOLATE LABEL**

**Figure 20 :- PALM JAGGERY CHOCOLATE**

## 6.6 PALM JAGGERY CHOCOLATE

- Nowadays, the demand for healthier sweeteners in chocolate (and foods in general), is increasing. Health issues related to high sugar levels and calories are a major concern.
- Palm sap sugar is claimed to be a healthier alternative sweetener to sucrose because it contains minerals and vitamins antioxidant and also exhibits low glycemic index (GI: 35-42) compared to pure sucrose (GI: 58-82)).



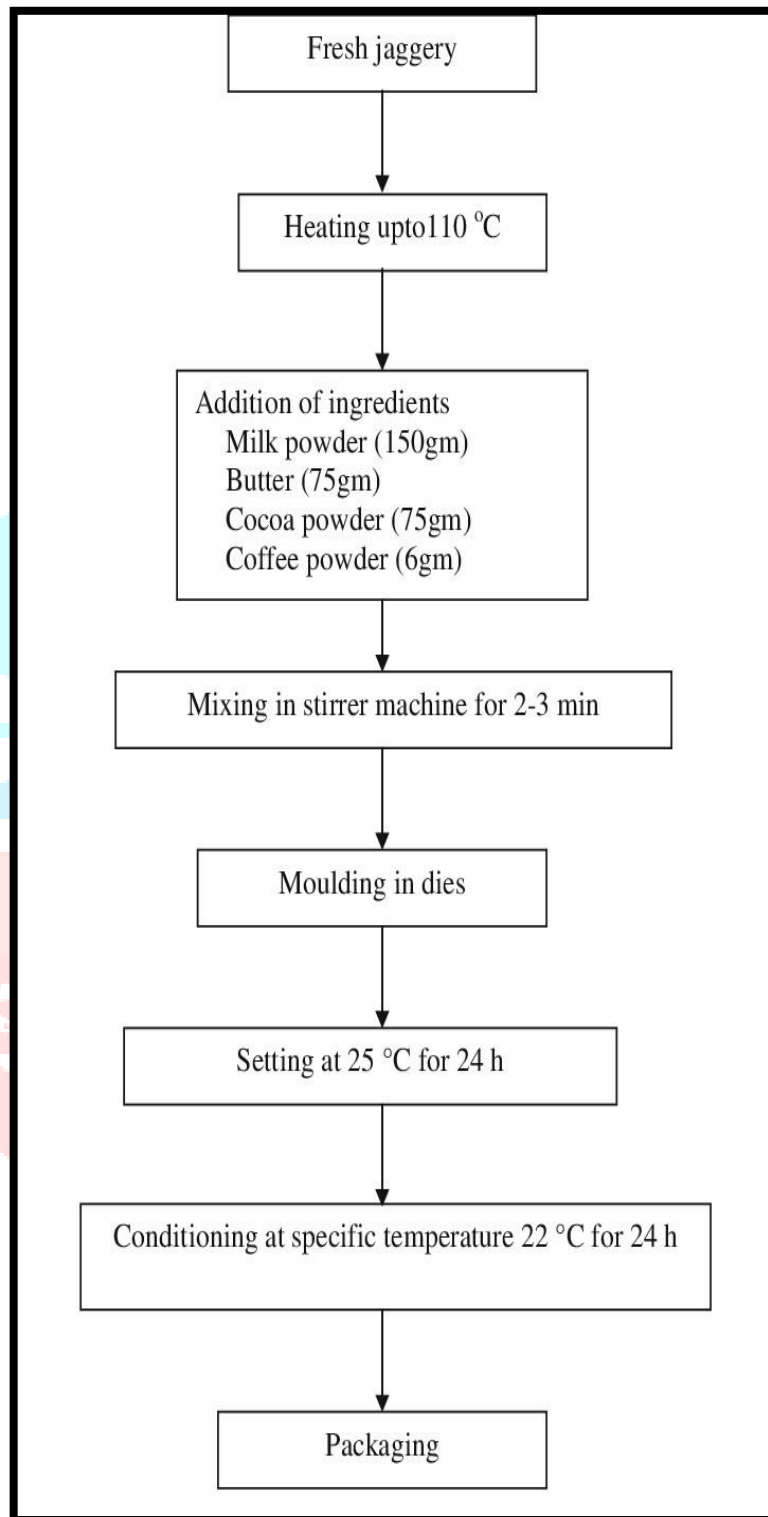
In addition, palm sap sugar also exhibits lower GI than that of a commercial milk chocolate (GI: 49), this type of sugar might be better consumed by people with high blood sugar levels. Moreover, it contains 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP). DDMP, which has a caramel-like flavour, is a Maillard reaction product which exhibits antioxidant activity and also has potency to reduce the risk for colon cancer.

- Because of the increasing demand for healthier chocolate, we utilised palm sap sugar to produce a healthier chocolate which does not only contain additional minerals, vitamins, anti-oxidative and anti-carcinogenic compounds compared to standard chocolate, but might also be beneficial for people with high blood sugar levels. [7, 18, 28,32,33]

**INGREDIENT: - Ingredients** used in preparation of palm jaggery chocolate

S. No	Ingredients(g)	Palm Jaggery chocolates (J2)
1	Palm Jaggery	49%
2	Cocoa mass	21%
3	Milk powder	12%
4	Xanthan gum	0.16%
5	Vallina essence	0.84%
6	Dates powder	17%

### 6.6.1 PROCESS: -



**Figure 7 :- Flow chart for preparation of palm Jaggery chocolates**



### **6.6.2 BENEFITS OF CHOCOLATE      PREPARED WITH PALM JAGGERY**

#### **AS SWEETENER**

Substitution of sucrose in dark chocolate with palm sap sugar has potential for the development of dark chocolate products with a distinctive flavour/aroma. Moreover, palm sap sugar sweetened chocolate, as a healthier chocolate, does not only contain additional minerals, vitamins, anti-oxidative and anti-carcinogenic compounds compared to standard chocolate, but may also be beneficial for diabetics. The aroma profile of palm sap sugar sweetened chocolate was marked with high concentrations of pyrazine-based compounds and also with the presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) which was not found in sucrose sweetened dark chocolate.

density and high moisture content of the palm sap sugars in combination with the



**Figure 22 :- PALM JAGGERY CHOCOLATE**



## **VII. EVALUATION PARAMETERS**

### **7.1 Organoleptic Evaluation**

Organoleptic evaluation means the study of drugs using organs of senses. It refers to the methods of analysis like colour, odour, taste, size, shape and special features, such as touch, texture, etc. Obviously, the initial sight of the sample is so specific that it tends to identify itself. If this is not enough, perhaps the sample has a characteristic odour or taste.

### **7.2 pH**

There are two methods for measuring pH: colorimetric methods using indicator solutions or papers, and the more accurate electrochemical methods using electrodes and a millivoltmeter (pH meter).

### **7.3 Melting point**

Palm jaggery or chocolate have very sharp and constant melting points.

### **7.4 Sucrose Identification:**

Dissolve 150.0 g in sufficient carbon dioxide-free water prepared from distilled water to produce 300 ml (solution A). Dilute 1 ml of solution A to 100 ml with water. To 5 ml of the solution add 2 ml of freshly prepared 2M sodium hydroxide and 0.15 ml of freshly prepared copper sulphate solution, the solution is clear and blue and remains so on boiling. To the hot solution add 4 ml of 2M hydrochloric acid, heat to boiling and add 4 ml of 2M sodium hydroxide; an orange precipitate is produced immediately.

### **7.5 Dextrose Assay:**

To an accurately measured volume equivalent to between 2 g and 5g of Dextrose, add 0.2 ml of 3M ammonia and sufficient water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube, Appendix 8.9. The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, C.H.O., in the volume taken for assay

### **7.6 DETERMINATION OF SUCROSE CONTENT:**

1. Three samples were prepared having different concentration of palm jaggery 25gm, 50gm, 100gm respectively in 10ml water and was observed under refractometer to check the sucrose content present in each sample.
2. A blank reading was taken using distilled water. The standard refractive index for distilled water is 1.33 and while observation our blank reading was 1.37. Then with three different samples three different readings were taken from refractometer scale.

3. Its procedure involves:

- I. Carefully place a small drop of jaggery solution on the hemicylinder.
- II. Apply the lock.
- III. Place a white light in front of the refractometer or using the natural source of light.
- IV. Look through the eyepiece and note the measurement on the scale.

### **7.7 Complexometric titration for calcium gluconate**

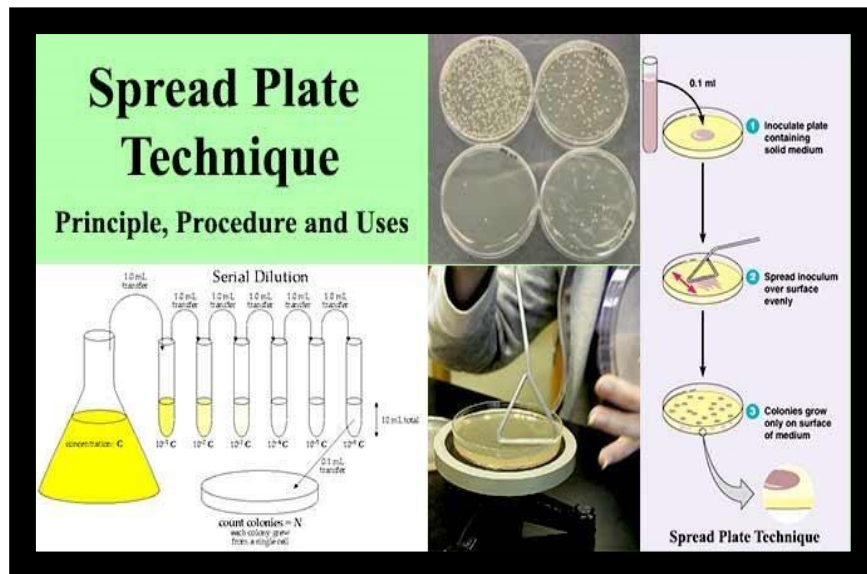
**Assay: -**

Weigh accurately about 0.5 g and dissolve in 50 ml of warm water; cool, add 5.0 ml of 0.05 M magnesium sulphate and 10 ml of strong ammonia solution and titrate with 0.05 M disodium edetate using mordant black H mixture as indicator. From the volume of 0.05 M disodium edetate required subtract the volume of the magnesium sulphate solution added. 1 ml of the remainder of 0.05 M disodium edetate is equivalent to 0.02242 g of  $\text{C}_{12}\text{H}_{22}\text{O}_{14}$ ,  $\text{H}_2\text{O}$ .

### **7.8 MICROBIAL ASSAY**

#### **SPREAD PLATE TECHNIQUE**

- This technique typically is used to separate microorganisms contained within a small sample volume, which is spread over the surface of an agar plate, resulting in the formation of discrete colonies distributed evenly across the agar surface when the appropriate concentration of cells is plated.
- In addition to using this technique for viable plate counts, in which the total number of colonies forming units on a single plate is enumerated and used to calculate the concentration of cells in the tube from which the sample was plated, spread-plating is routinely used in enrichment, selection, and screening experiments.
- The desired result for these three experiments is usually the same as for plate counts, in which a distribution of discrete colonies forms across the surface of the agar. However, the goal is not to ensure *all* viable cells form colonies. The spread plate procedure may be employed over the pour plate technique for an enumeration experiment if the end goal is to isolate colonies for further analysis because colonies grow accessibly on the agar surface whereas they become embedded in the agar with the pour plate procedure.



**Figure 8:- SPREAD PLATE TECHNIQUE**

✚ There are two strategies described here for the spread plate procedure

- The first (Method A) involves use of a turntable and glass or metal rod shaped like a hockey stick.
- The second (Method B), referred to as the "Copacabana Method", involves shaking presterilized glass beads. Both facilitate even spreading of cells across the agar surface.

### **PROCEDURE: -**

1. Label around the edge of the bottom (not the lid) of an agar plate with at least your name, the date, the type of growth medium, and the type of organism to be plated on the medium.
  - Include the dilution factor if plating serial dilutions.
  - The plates must be completely dry without condensation on the lid and pre-warmed to room temperature prior to spread-plating. If the plates are stored at 4 °C, remove them several hours or even the day before. Spread them out in small, staggered stacks of no more than 2-3 plates and allow them to dry.
2. Center the plate on the turntable.

3. Obtain your sample, which should be a broth culture or a suspension of cells produced by mixing cells from a colony into buffer or saline.
  - The samples may be derived from a dilution series of a single sample.
  - Sample volume to be plated should be between 0.1 and 0.2 ml.
4. Open the lid of the Petri dish, and dispense your sample onto the center of the agar. Close the lid.
  - Use aseptic technique throughout this procedure.
  - Use a micropipettor to transfer your sample to the plate. Control the flow of the sample so it does not splash out of the plate.
5. Dip the glass rod or metal rod (also called a spreader) into a beaker of 70% (v/v) ethanol.
  - CAUTION: Never dip a hot spreader into a beaker of alcohol.
  - The ethanol must only touch the bottom portion of the spreader and the first inch of the stem.
6. Drain and ignite excess ethanol by passing it through the flame of a Bunsen burner.
  - The flame should travel the length of the spreader and stem that came in contact with the ethanol then quickly extinguish.
  - Should the beaker of ethanol catch fire, do not panic! Place a glass cover over the beaker, which will quickly extinguish the fire.
7. Open the lid of the agar plate, holding the lid in your left hand with your thumb and index finger. Cool the spreader by touching it to the agar along the edge near the rim.
  - Do not touch the agar where the cells were added. The hot spreader will kill the cells.
8. With your left hand (while still holding the lid of the agar plate), spin the turntable slowly.
  - Although best avoided, if you must put the lid down, place it face down on a disinfected surface within the sterile field of the Bunsen burner. With a lid that faces up, there is a greater chance of contamination from movements of objects or hands, creating air currents that cause microorganisms and dust particles to descend to the inside surface of the lid.

9. With your right hand, hold the spreader gently on the surface of the agar and gradually spread the sample evenly over the entire plate. Move the spreader back and forth across the plate as the turntable is spinning.
10. Allow the sample to absorb thoroughly (at least 5 minutes) before inverting the plate for incubation.



**Figure 9:- SPREAD PLATE METHOD**

## **VIII. RESULTS AND DISCUSSION**

### **7.1 BASIC ORGANOLEPTIC EVALUATION TESTS**

#### **○ FOR RAW PALM JAGGERY**

Sr. no	Analytical methods	Result
1	Colour	Brown
2	Odour	Pleasant, Aromatic
3	Taste	Sweet
4	Texture	Crystalline, Granuals
5	pH	6
6	Melting profile	200 <sup>0</sup> C
7	Solubility	Soluble in water

#### **○ FOR CHOCOLATE**

Sr. no	Analytical methods	Result
1	Colour	Brown to Dark brown
2	Odour	Pleasant, Aromatic
3	Taste	
4	Texture	Smooth and Soft
5	pH	6
6	Melting profile	30 <sup>0</sup> C – 32 <sup>0</sup> C
7	Solubility	Soluble in water

#### **○ pH**

Sr. no	Sample	Readings
1	Raw palm jaggery	6
2	Palm jaggery chocolate	5.6

### ○ DETERMINATION OF SUCROSE CONTENT

Sr. no	Sample	Readings
1	Sample 1 (25mg)	1.336
2	Sample 2 (50mg)	1.336
3	Sample3 (100mg)	1.339

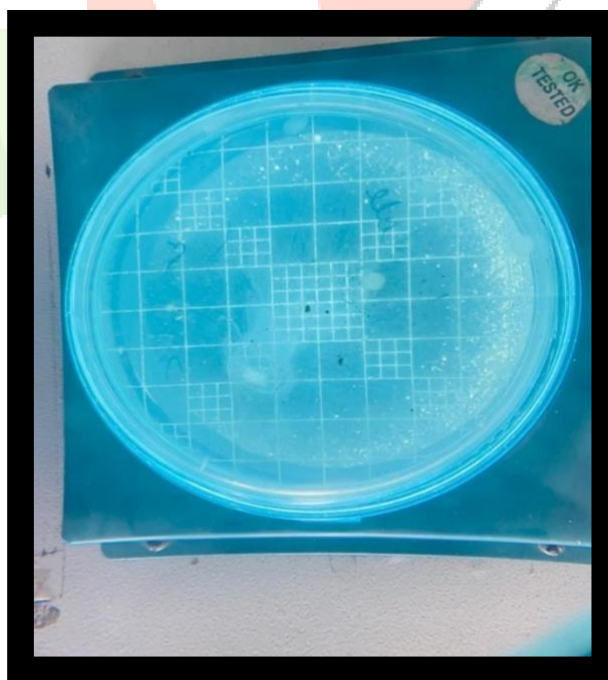
### ○ DETERMINATION OF DEXTROSE CONTENT

Sr no	SAMPLE	READINGS
1	WATER	126.55
		226.55
2	SAMPLE (DARK CHOCOLATE)	330.75
		331.23

### ○ MICROBIAL LOAD

After incubation the colonies on the agar plate are observed. Some of the colonies are free from each other.

Visible isolated colonies of bacteria that are distributed on plate and are countable.



**Figure 25 VISIBLE BACTERIA COLONIES**



## **IX. CONCLUSION**

- The use of palm sap sugar instead of sucrose in dark chocolate could lead to the development of dark chocolate products with distinct flavours and aromas. Furthermore, palm sap sugar-sweetened chocolate, as a healthier chocolate, not only contains more minerals, vitamins, anti-oxidative, and anti-carcinogenic substances than ordinary chocolate, but it may also benefit diabetics. The presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) in palm sap sugar sweetened dark chocolate was not identified in sucrose sweetened dark chocolate.
- The lighter colour, higher hardness, and higher viscosity of the palm sap sugar-sweetened chocolates were due to the low particle density and high moisture content of the palm sap sugars, combined with the presence of reducing sugar. Palm sap sugar, with additional advancements in processing technology, can serve as a better and healthier alternative to standard sucrose, resulting in chocolates with greater health advantages for health-conscious consumers and the general public. Chocolates made with palm jaggery contain more protein than conventional chocolates. In the sensory evaluation process, the most acceptable formulation in, which contains 50 percent palm jaggery and has the best overall acceptability results.
- 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), an anti-oxidant and anticarcinogenic molecule, is present in chocolate sweetened with palm sugar regardless of the processing method.
- Palm jaggery has glycaemic index score is 41, which is appropriate for diabetes too. However, it should be consumed in moderation as it is high in sugar while Normal white sugar has Glycaemic index score up to 68-70.
- Sugar, on the other hand, is made up entirely of sucrose. Coconut sugar is better for you because fructose and glucose are rapidly absorbed and transformed into energy, whereas sucrose must first be broken down into monosaccharides. Coconut sugar, which contains Cu, Fe, Zn, and Mn, has a complete metal content. Fe, Zn, and Cu are all present in palm sugar, however Fe and Zn are only present in refined Granulated Sugar. Coconut sugar and palm sugar have a high metal level of Fe, which helps to prevent anaemia

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